

# Initial Genome Scan of the NIMH Genetics Initiative Bipolar Pedigrees: Chromosomes 1, 6, 8, 10, and 12

John P. Rice,<sup>1\*</sup> Alison Goate,<sup>1</sup> Jeff T. Williams,<sup>1</sup> Laura Bierut,<sup>1</sup> David Dorr,<sup>1</sup> William Wu,<sup>1</sup> Shantia Shears,<sup>1</sup> Gayathri Gopalakrishnan,<sup>1</sup> Howard J. Edenberg,<sup>2</sup> Tatiana Foroud,<sup>2</sup> John Nurnberger, Jr.,<sup>2</sup> Elliot S. Gershon,<sup>3</sup> Sevilla D. Detera-Wadleigh,<sup>3</sup> Lynn R. Goldin,<sup>3</sup> Juliet J. Guroff,<sup>3</sup> Francis J. McMahon,<sup>4</sup> Sylvia Simpson,<sup>4</sup> Dean MacKinnon,<sup>4</sup> Melvin McInnis,<sup>4</sup> O. Colin Stine,<sup>4</sup> J. Raymond DePaulo,<sup>4</sup> Mary C. Blehar,<sup>5</sup> and Theodore Reich<sup>1</sup>

<sup>1</sup>Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri

<sup>2</sup>Department of Biochemistry and Molecular Biology, Institute for Psychiatry Research, Indiana University Medical Center, Indianapolis, Indiana

<sup>3</sup>Clinical Neurogenetics Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

<sup>4</sup>Department of Psychiatry, Johns Hopkins University Medical School, Baltimore, Maryland

<sup>5</sup>Mood, Anxiety, and Personality Disorders Research Branch, NIMH, NIH, Rockville, Maryland

A report on an initial genome screen on 540 individuals in 97 families was collected as part of the NIMH Genetics Initiative on Bipolar Disorder. Families were ascertained to be informative for genetic linkage and underwent a common ascertainment and assessment protocol at four clinical sites. The sample was genotyped for 65 highly polymorphic markers from chromosomes 1, 6, 8, 10, and 12. The average intermarker interval was 16 cM. Genotypic data was analyzed using affected sib pair, multipoint affected sib pair, and pedigree analysis methods.

Multipoint methods gave lod scores of approximately two on chromosomes 1, 6, and 10. The peak lod score on chromosome 6 occurred at the end of the q-arm, at some distance from the 6p24-22 area previously implicated for schizophrenia. We are currently genotyping additional markers to reduce the intermarker interval around the *signals*.

The interpretation of results from a genome screen of a complex disorder and the problem of achieving a balance between detecting

false positive results and the ability to detect genes of modest effect are discussed. *Am. J. Med. Genet.* 74:247–253, 1997.

© 1997 Wiley-Liss, Inc.

**KEY WORDS:** bipolar disorder; linkage analysis; genome scan; genome screen; affected sib pairs

## INTRODUCTION

There is convincing evidence that susceptibility towards bipolar affective disorder is transmitted through genetic factors [see Craddock and McGuffin, 1993; Sham et al., 1992], but the basic questions of mode of inheritance and identification of particular susceptibility loci remain largely unanswered [Baron, 1991]. Several genomic regions have been implicated as *candidate* regions during the past twenty years, but many have failed to replicate [see Risch and Botstein, 1996]. The rate of discovery of possible loci has increased with polymorphic DNA marker and polymerase chain reaction (PCR) technology, leaving us with regions of chromosomes 4, 5, 6, 11, 12, 13, 15, 16, 18, 21, and X implicated at one time or another. Current interest focuses on chromosomes 4, 18, and 21 [Detera-Wadleigh et al., 1997].

The NIMH Genetics Initiative for Bipolar Disorder was originally designed to provide a large sample of genetically informative bipolar pedigrees and associate DNA for study by the scientific community [Blehar et al., 1988]. We report on the genetic analysis of 97 families selected to have at least one affected sib pair; we also implement some recent improvements in statistical methodology. The Washington University Center is

Contract grant sponsor: USPHS; Contract grant numbers: MH46280, MH37685, and MH31302.

Material presented here does not necessarily reflect the opinions, official policy, or position of the National Institute of Mental Health.

\*Correspondence to: John P. Rice, Ph.D., Department of Psychiatry (Box 8134), Washington University School of Medicine, 4940 Children's Place, St. Louis, Missouri 63110-1093. Email: john@jpr.wustl.edu

Received 7 January 1997; Revised 12 March 1997

mapping chromosomes 1, 6, 8, 10, and 12. Previous work has indicated potential linkage to several regions on these chromosomes. Craddock et al. [1994] has shown co-segregation between the Darier's disease gene locus and bipolar disorder at 12q23-q24.1 in a single family in Great Britain. This finding was initially refuted as being a bipolar-only locus by Ewald et al. [1994] using two families showing susceptibility to bipolar disorder but no history of Darier's. However, subsequent analyses [Dawson et al., 1995a; Dawson et al., 1995b] using 45 families with only bipolar disorder showed modest evidence for linkage to the Phospholipase A2 gene locus. An analysis of fragile sites in bipolar and unaffected individuals showed a higher frequency of a fragile site at 1q32 in the bipolar population compared to controls using low folate media [Turecki et al., 1995]. Despite the low power of the study (10 affected vs. 10 controls), the rarity of this particular cytogenetic marker and previous evidence for cytogenetic abnormalities in transmission of bipolar illness [Craddock et al., 1993] provide an impetus for a closer examination of such abnormalities.

Taylor et al. [1993] reported an increased risk of affective disorder in relatives of schizophrenics. Chromosome 6q24-22 has been implicated in the predisposition of schizophrenia [Straub et al., 1995; Wang et al., 1995; Schwab et al., 1995], with the strongest evidence for linkage found in 6p23-loci D6S260-D6S274-D6S285, a region of about 5 cM.

We report here the results from our initial genome screen of chromosomes 1, 6, 8, 10, and 12. These analyses use multiple perspectives (affected sib pairs, entire pedigrees, multiple diagnostic schema and multipoint affected sib pair methods) to identify *hot spots* for further study. The initial screen has generated a 20 cM map, with the next step being the creation of a high density map in the targeted regions to permit multipoint analyses. The overall design of the study is presented in this issue [NIMH Genetics Initiative Bipolar Group et al., 1997], as well as the results from the initial screen of the other chromosomes [Detera-Wadleigh et al., 1997; Stine et al., 1997; Edenberg et al., 1997].

## MATERIALS AND METHODS

### Genotyping

The microsatellite polymorphisms were detected by PCR using oligonucleotide primers. The majority of markers were tetranucleotide repeat polymorphisms from the CHLC maps. Dinucleotide polymorphisms from Utah and the Genethon maps were used only when gaps of >20 cM existed in the tetranucleotide repeat map. The forward primers were labelled at the 5' end with a 6-carboxyfluorescein known as 6-FAM, tetrachlorinated analogue (TET), or hexachlorinated analogue (HEX) (Applied Biosystems). The markers were carefully selected so that each chromosome set could be multiplexed on single ABI gel (in the case of chromosome 1 two sets were required to cover the chromosome). The 5-ml PCR reactions contained 100 ng template DNA, 3.5 pmol end-labeled forward primer and unlabeled reverse primer, 0.2 mM dNTPs, 1 u Taq DNA polymerase (Perkin-Elmer) and 1 ml 5X buffer

(7.5 mM MgCl, 250 mM KCl, 50 mM Tris HCl pH8.3). All reaction cocktails were aliquoted into a 96-well Falcon assay plate using a Beckman-1000 Workstation. PCR reactions were carried out on a Hybrid OmniGene thermal cycler using the following cycling conditions: initial denaturation at 96°C for 4 min, followed by optimized (22-28) cycles of 94°C for 1 min, optimized annealing temperature (56°-62°C) for 1 min, and 72°C for 45 sec, and a final extension of 5 min at 72°C. The PCR products were then diluted eight-fold using the Beckman Workstation. 0.8 ml of diluted PCR product was mixed with 2.5 ml of deionized formamide, 0.4 ml internal lane (size) standard (TAMRA-350, Applied Biosystems) and 0.5 ml loading dye, denatured at 97°C for 5 min, rapidly cooled on ice and then electrophoresed on a 6% denaturing polyacrylamide gel. The alleles were detected on an ABI 373 automated DNA sequencer using 672 GENESCAN software. Control samples were loaded on every gel. All alleles were initially genotyped semiautomatically using GENOTYPER software (Applied Biosystems).

Estimated base pair sizes are given to two decimal places. The simple rounding of these sizes is problematic due to gel-dependent shifts [Rice et al., 1995], so heterozygous controls were used on each gel with each marker to permit standardization across gels.

We used scoring algorithms which are part of the database system GENEMASTER on a SUN Workstation. Data were first transferred from the lab Macintosh to the SUN, and entered into the system. A shift constant for each family was computed so that genotypes round to appropriate values (integers which are 2, 3, or 4 apart for di-, tri-, and tetranucleotide repeats, respectively). Once the genotypes were scored, they were again shifted so that the standard on each gel coincided with control values. Both the initial genotype and data flagged as non-inheritance errors were rechecked manually with GENOTYPER and CRIMAP software in order to minimize the error rate.

### Marker Frequencies and Map

Once the marker data were clean, the allele frequencies were estimated using the program USERM13 [Boehnke, 1991] and entered into the GENEMASTER database. For a given chromosome, the marker order was specified and the program CRIMAP used to estimate the (sex-averaged) distances given in Tables I through V. A Kosambi map function was assumed in these computations.

### Subjects and Diagnoses

A total of 97 families were included in the genetic analyses. There were 623 individuals with final best estimate diagnoses and 540 individuals with DNA available for genotyping in these families. In the sample of 540 genotyped individuals, 32 had a diagnosis of schizoaffective bipolar (SA/BP), 232 of bipolar I (BPI), 72 of bipolar II (BP II), 88 of recurrent major depressive disorder (UPR), 71 with other diagnoses, and 45 were never mentally ill (NMI). These families were chosen to be dense, informative families for linkage linkage. Parents were included when DNA was

available. The families averaged 5.6 individuals genotyped, ranging from 1 family of 2 members to 2 families with 14 each. Details on the ascertainment process and diagnostic procedures are provided elsewhere in this issue.

Three parallel sets of analyses were performed under these diagnostic schema—Models I to III. Under Model I individuals were classified as affected if they had a lifetime diagnosis of SA/BP or BPI. Model II also included as affected individuals those with a diagnosis of BPII, and Model III included individuals with UPR. Individuals who were NMI were included as unaffected, and all others were coded as *unknown*. The number of affected sib pairs for a particular marker is given in Tables I through V. These are calculated as all possible affected sib pairs when more than two affected siblings are part of a nuclear family.

### Affected Sib Pair Analyses

The affected sib pair analyses [Blackwelder and Elston, 1985] used version 2.7.2 of SIBPAL, part of release 2.2 of S.A.G.E. (Statistical Analysis for Genetic Epidemiology) [1994]. The mean identity by descent (IBD) is computed and compared to the value of one half expected for full siblings under the hypothesis of no linkage. This method uses all genotypic information to estimate IBD, but only uses affected sib pairs as the unit of analysis. In this version of SIBPAL, separate tests are performed for full-sib and half-sib pairs. We report on only the test based on full siblings. When parents are untyped, the gene frequencies of the marker alleles are used to estimate IBD.

### MOD Score Analysis

We also used a technique introduced by Risch [1984], referred to as the MOD score method. This method uses the likelihood of marker data conditioned on all trait phenotypes:

$$L = P(\text{marker data} | \text{all trait phenotypes})$$

It notes that this formulation obviates the need for systematic ascertainment of pedigrees and provides information on the mode of inheritance only via the linkage information. Risch viewed this as an extension of the affected sib pair method to arbitrary pedigrees.

Clerget-Darpoux et al. [1986] introduced the term *MOD score* the model lod score, computed by maximizing the lod score under the assumption that the trait is determined by a single diallelic locus with recombination fraction  $q$  and parameters  $f = (p, f_1, f_2, f_3)$ , where  $p$  is the gene frequency and  $f_1, f_2, f_3$  are the three penetrances associated with the trait locus. Elston [1989] noted the equivalence of this method to that of Risch when the trait is controlled by a single locus.

We used the entire pedigree for the MOD score analysis and the program MODLINK [Rosalind Neuman, personal communication], a modified version of ILINK that maximizes the lod score rather than the likelihood. We multiply the MOD score by 4.6 and compare that value to a chi-square with three degrees of freedom. One of the major differences from affected sib pair analyses is that a pedigree with multiple affected sibs is analyzed jointly.

## Multipoint Sib Pair Analysis

The program ASPEX [David Hinds and Neil Risch, unpublished] was used for multipoint sib pair analyses. This program uses marker information from all sibs in the sibship but examines IBD status for individual sib pairs. We used the *sib\_ibd* program which does not use marker allele frequencies to infer IBD and examined all possible  $n(n-1)/2$  pairs in a nuclear family with  $n$  affected sibs. Extended pedigrees were broken up into their constituent nuclear families. All figures present the results from the multipoint sib pair analysis for Models II and III.

## RESULTS

In Tables I through V and Figures 1 through 5 we display results using SIBPAL, MODLINK, and ASPEX. The number of the affected individuals available in analysis varies by the marker as indicated in the Tables. This is due to missing genotypic data. There are potentially 121, 197, and 282 affected sib pairs for Models I, II, and III, respectively. The columns labeled N-pairs refer to the number of pairs where both members have genotypic data. If, for example, 90% of genotypes can be scored for a particular marker, then we would expect 81% of sib pairs to have both members with complete data. This underscores one advantage of the multipoint analysis since individuals with missing genotypic data will likely be genotyped at flanking markers.

### Chromosome 1

We used a total of 21 markers on chromosome 1 (Table I). Allele sharing was found to be 54% to 55% for D1S224 under all three diagnostic schema and 53% for D1S1648 for Model III. The highest MOD score was 1.94 for D1S1648. As noted in Figure 1, the multipoint analyses had a distinct peak between D1S224 and D1S1648. These two markers are 10 cM apart. Additional markers will be needed to investigate this area further.

### Chromosome 6

We examined 11 markers on chromosome 6. Excess sharing and significant MOD scores were noted between D6S474-D6S495. As noted in Figure 2, the maximal evidence is at D6S1009. Each individual marker reaches significance at  $0.025 < P < 0.05$  under only the broadest diagnostic schema, Model III.

### Chromosome 8

We examined nine markers on chromosome 8. Although several markers gave significant results under affected sib pair analyses, only one of these gave a significant MOD score. The significant signals were punctuated with markers with non-significant sharing. This is reflected in the modest multipoint lod scores (Fig. 3).

### Chromosome 10

We examined 13 markers on chromosome 10 (Table IV). One marker (D10S188) gave excess sharing and significant MOD scores of 3.41, 3.47, and 2.35 for Models I through III, respectively. The flanking markers

TABLE I. Analytic Results Under Three Diagnostic Schema for Chromosome 1

Locus	Dist (cM)	Model I			Model II			Model III		
		N-pairs <sup>a</sup>	IBD <sup>a</sup>	MOD <sup>b</sup>	N-pairs	IBD	MOD	N-pairs	IBD	MOD
D1S548	0.0	106	.49	0.21	171	.50	0.79	251	.50	1.34
D1S1612	10.5	73	.47	0.86	124	.46	0.90	185	.48	0.76
D1S1592	32.4	98	.48	0.00	157	.48	0.00	232	.48	0.07
D1S1598	55.2	102	.47	0.00	170	.48	0.02	249	.48	0.05
D1S1596	78.0	101	.51	0.65	164	.51	1.14	245	.51	1.02
D1S1613	86.7	74	.51	0.12	110	.51	0.18	150	.51	0.00
D1S224	96.2	83	.54*	0.71	138	.55***	1.66	198	.54**	1.65
D1S1648	106.2	108	.49	0.54	172	.51	0.88	245	.53***	1.94*
D1S550	116.4	89	.55*	1.30	151	.54*	0.75	217	.52	0.64
D1S532	121.3	90	.50	0.86	136	.46	1.16	196	.48	0.00
D1S1588	140.7	95	.51	1.14	158	.53	1.37	233	.52	0.65
D1S1631	155.6	79	.47	0.05	130	.50	0.91	197	.51	0.97
D1S1675	166.7	95	.51	0.02	162	.52	0.84	237	.51	1.05
D1S534	174.7	88	.48	0.00	137	.51	0.62	190	.52	1.50
D1S1595	185.1	107	.52	1.10	172	.51	0.91	255	.50	1.10
D1S1518	217.7	83	.47	0.92	134	.48	0.54	196	.48	0.27
D1S1606	232.0	96	.51	0.66	154	.50	0.48	232	.51	0.42
D1S1602	250.8	93	.46	0.02	148	.46	0.06	214	.47	0.05
D1S2141	252.4	89	.45	1.44	146	.45	0.28	214	.47	0.59
D1S549	260.6	104	.45	0.36	171	.47	0.57	248	.46	0.00
D1S547	281.0	77	.50	0.01	130	.49	0.01	191	.49	0.00

<sup>a</sup>Number of affected sib pairs (N-pairs) and estimated IBD from SIBPAL program.

<sup>b</sup>Maximum lod score using entire pedigrees.

\* $P < .05$ , \*\* $P < .025$ , \*\*\* $P < .01$ .

TABLE II. Analytic Results Under Three Diagnostic Schema for Chromosome 6

Locus	DIST (cM)	Model I			Model II			Model III		
		N-pairs <sup>a</sup>	IBD <sup>a</sup>	MOD <sup>b</sup>	N-pairs	IBD	MOD	N-pairs	IBD	MOD
D6S477	0.0	110	.53	1.53	182	.52	1.46	260	.51	1.16
D6S1006	16.6	112	.50	0.00	177	.48	0.00	257	.50	0.00
D6S1019	43.4	107	.47	0.00	177	.49	0.00	255	.50	0.19
D6S1018	53.9	115	.50	0.38	185	.51	0.77	260	.51	0.51
D6S493	69.1	85	.48	0.49	135	.49	0.38	197	.51	0.20
D6S1082	87.0	95	.47	0.05	145	.51	0.53	205	.51	1.17
D6S474	115.3	106	.54	1.74*	167	.54	1.38	236	.53	1.27
D6S1009	142.1	66	.54	0.33	99	.54	1.53	127	.55*	2.08*
D6S1961	156.8	106	.52	0.69	161	.51	0.57	224	.54*	1.05
D6S495	170.0	114	.52	1.25	176	.51	1.29	244	.52	2.37*
D6S1007	184.8	110	.53	1.69	171	.52	1.57	243	.53	1.25

<sup>a</sup>Number of affected sib pairs (N-pairs) and estimated IBD from SIBPAL program.

<sup>b</sup>Maximum lod score using entire pedigrees.

\* $P < .05$ .

TABLE III. Analytical Results Under Three Diagnostic Schema for Chromosome 8

Locus	DIST (cM)	Model I			Model II			Model III		
		N-pairs <sup>a</sup>	IBD <sup>a</sup>	MOD <sup>b</sup>	N-pairs	IBD	MOD	N-pairs	IBD	MOD
D8S1099	0.0	109	.50	0.32	167	.53	1.27	234	.51	1.47
D8S1106	10.5	109	.50	0.00	175	.53*	2.01*	251	.54**	1.65
D8S1145	24.0	106	.47	0.04	170	.50	0.25	240	.51	0.62
D8S1128	52.0	104	.52	0.23	163	.52	0.37	227	.52	1.55
D8S1102	66.7	108	.51	0.33	170	.52	0.69	241	.52	0.87
GATA12B06	79.2	102	.54	1.27	163	.54**	1.73*	232	.52	0.97
D8S1119	93.0	105	.50	0.05	165	.52	0.10	240	.50	0.48
GAAT1A4	103.8	110	.48	0.08	175	.49	0.35	245	.48	0.00
D8S588	117.4	99	.47	0.00	162	.48	0.00	232	.49	0.00

<sup>a</sup>Number of affected sib pairs (N-pairs) and estimated IBD from SIBPAL program.

<sup>b</sup>Maximum lod score using entire pedigrees.

\* $P < .05$ , \*\* $P < .025$ .

TABLE IV. Analytical Results Under Three Diagnostic Schema for Chromosome 10

Locus	DIST (cM)	Model I			Model II			Model III		
		N-pairs <sup>a</sup>	IBD <sup>a</sup>	MOD <sup>b</sup>	N-pairs	IBD	MOD	N-pairs	IBD	MOD
D10S249	0.0	105	.51	0.51	170	.52	0.85	245	.50	0.76
D10S591	11.6	92	.50	0.02	147	.50	0.30	217	.50	0.29
D10S547	24.4	79	.51	0.81	137	.50	0.56	211	.51	1.02
D10S1423	54.2	95	.57***	2.04**	141	.57***	3.40***	203	.53	1.77*
D10S1426	65.9	85	.51	0.78	139	.54*	2.06**	193	.52	2.07**
D10S539	80.9	109	.50	0.69	179	.49	0.25	254	.50	0.73
D10S1211	97.0	103	.50	0.40	166	.50	1.11	239	.50	0.22
D10S188	115.2	110	.55*	3.41***	182	.54**	3.47***	261	.52	2.35**
D10S222	158.4	101	.52	0.27	166	.50	0.07	246	.50	0.00
D10S221	182.4	100	.48	0.09	161	.50	0.00	232	.50	0.00
D10S610	190.9	75	.51	0.31	125	.53	0.53	192	.54*	1.47
D10S186	2229.2	94	.49	0.19	156	.49	0.01	218	.50	0.44
D10S212	245.8	113	.48	0.04	184	.53	1.32	264	.52	1.33

<sup>a</sup>Number of affected sib pairs (N-pairs) and estimated IBD from SIBPAL program.

<sup>b</sup>Maximum lod score using entire pedigrees.

\* $P < .05$ , \*\* $P < .025$ , \*\*\* $P < .01$ .

are approximately 20 cM and 40 cM on either side of D10S188, so the multipoint results (Fig. 4) are not useful for this chromosomal region. A second marker (D10S1423) was also significant, and additional markers will be placed in the 30 cM gap between D10S547 and D10S1423.

### Chromosome 12

We examined 11 markers on chromosome 12 (Table V). There was an isolated signal at D12S379 with a maximal MOD score of 1.89 under Model III. The sib pair results were not significant at this marker. The multipoint analysis (Fig. 5) gave a minimal signal near this locus. Neither the sib pair nor the MOD score analysis gives significant results near D12S1090 where the multipoint is maximized.

### Summary of Results

There were modest *signals* on chromosomes 1, 6, and 10. There were two signals on chromosome 10 where both the affected sib pair analyses and the MOD scores were significant at the  $P = 0.01$  level. However, the multipoint analysis had its highest peak (lod of approximately 2) in Figure 4 in the 40 cM gap between

markers. The signals on 10 were significant under all three diagnostic schema and showed the most haplotype sharing under the narrowest criteria. The MOD score is more directly related to statistical significance, and the 54% sharing in 182 pairs is more significant than the 55% sharing in the 110 pairs in Table IV for D10S188.

In all cases, a denser map in these areas of interest is required before drawing any firm conclusions from this study. These results, or results from any single study, should be interpreted with caution.

### DISCUSSION

There is some evidence for linkage on our particular chromosomes, but this evidence is not overwhelming. As noted in the introduction there are some prior reports of linkage to markers on our chromosomes. The peak on chromosome 1 (D1S224, D1S1648) was localized between 1p22.3-1p21. However, we detected no signal with three markers (D1S1602, D1S2141, D1S549) near the fragile site region (1q32) identified by Turecki et al. [1995].

The signal on chromosome 6 was limited to the q arm of the chromosome, and thus does not add to the hy-

TABLE V. Analytic Results Under Three Diagnostic Schema for Chromosome 12

Locus	DIST (cM)	Model I			Model II			Model III		
		N-pairs <sup>a</sup>	IBD <sup>a</sup>	MOD <sup>b</sup>	N-pairs	IBD	MOD	N-pairs	IBD	MOD
D12S372	0.0	92	.51	0.15	146	.48	0.44	207	.50	0.47
D12S397	12.4	81	.47	0.00	116	.50	0.24	178	.51	0.00
D12S373	32.2	60	.48	0.00	92	.49	0.00	125	.53	0.53
D12S1090	51.2	68	.49	1.19	109	.51	0.36	152	.53	0.42
D12S390	64.7	105	.49	0.34	153	.49	0.26	204	.50	0.40
D12S375	80.8	94	.54	1.38	144	.52	0.84	214	.52	0.79
D12S379	101.7	81	.51	0.88	118	.54	1.51	173	.52	1.89*
D12S393	109.6	118	.52	0.27	183	.52	0.53	262	.51	0.36
D12S395	138.5	101	.48	0.00	158	.52	1.06	231	.52	0.66
D1S1045	168.2	83	.47	0.07	137	.48	0.00	204	.49	0.00
D12S392	175.3	116	.48	0.01	178	.48	0.01	239	.50	0.12

<sup>a</sup>Number of affected sib pairs (N-pairs) and estimated IBD from SIBPAL program.

<sup>b</sup>Maximum lod score using entire pedigrees.

\* $P < .05$ .

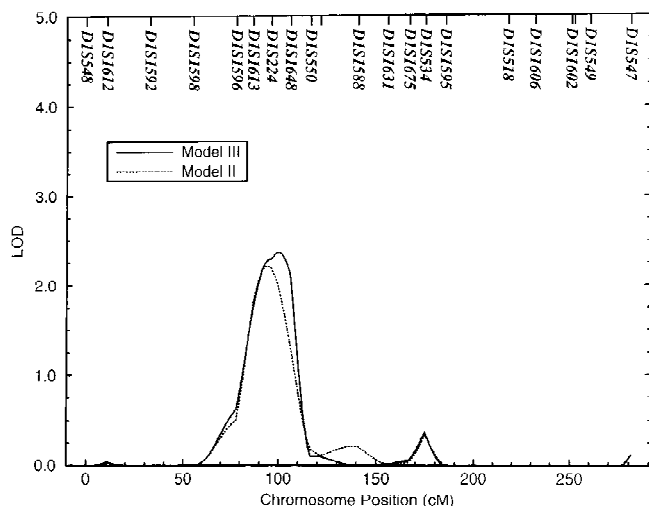


Fig. 1. Multipoint lod score for chromosome 1. Results are computed using IBD information on all possible pairs of affected siblings for diagnostic Models II (schizoaffective bipolar, bipolar I and bipolar II) and III (Model II plus recurrent unipolar individuals).

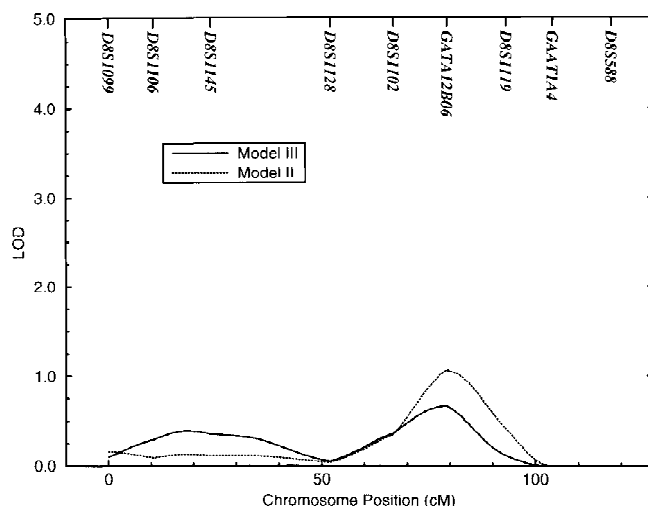


Fig. 3. Multipoint lod score for chromosome 8. Results are computed using IBD information on all possible pairs of affected siblings for diagnostic Models II (schizoaffective bipolar, bipolar I and bipolar II) and III (Model II plus recurrent unipolar individuals).

pothesis of a multidisorder locus near 6p24-22. One of our markers (D6S1006) localized within 3 cM of D6S259, a marker 5 cM proximal to the hot locus around D6S274 [see Straub et al., 1995; Schwab et al., 1995], and another (D6S1019) appeared 10 cM telomeric of the locus. None of these gave a signal.

The link between chromosome 12 and bipolar disorder independent of Darier's disease is still in contention. The MOD score for the broadest diagnostic category, Model III, was significant ( $P < .05$ ) at marker D12S379 near 12q21. This has been localized about 20 cM away from the D12S79-D12S84-PLA2A locus indicated by Dawson et al. [1995a]. However, a marker closer to that region (D12S393, about 10 cM away) shows no signal in our data. Recently, LaBuda et al. [1996] reported suggestive results in the region 12q12-

24.1 in the Old Order Amish. They used IBS methods in affected relative pairs and found D12S18 and D12S14 to be each significant and an extended haplotype analysis showed a common haplotype in 63% of affected individuals.

The interpretation of our results, or results from any genome screen of a complex disorder, presents many challenges. For a Mendelian trait, the  $P$ -value corresponding to a lod score of 3 is approximately 0.0001 [Ott, 1991]. This has led to a high positive predictive value for *traditional* linkage studies. To maintain the comparable specificity for a complex trait, this cut-off would have to be much higher. This would, however, reduce the sensitivity to identify genes of modest effect. This dilemma is only partly resolved through replication studies. For example, if a disease had six suscep-

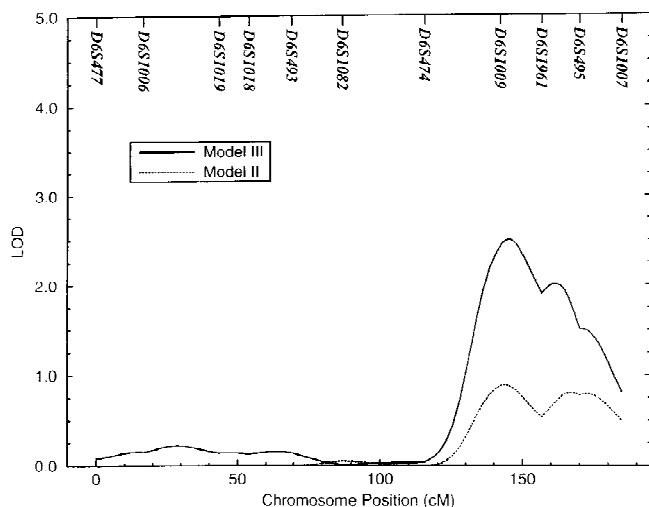


Fig. 2. Multipoint lod score for chromosome 6. Results are computed using IBD information on all possible pairs of affected siblings for diagnostic Models II (schizoaffective bipolar, bipolar I and bipolar II) and III (Model II plus recurrent unipolar individuals).

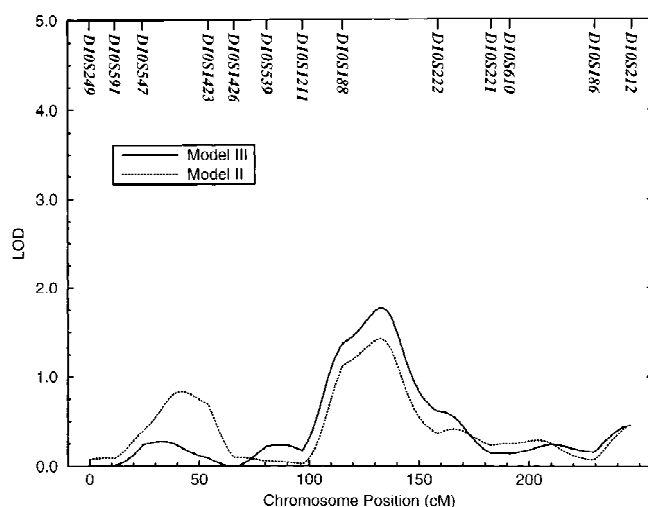


Fig. 4. Multipoint lod score for chromosome 10. Results are computed using IBD information on all possible pairs of affected siblings for diagnostic Models II (schizoaffective bipolar, bipolar I and bipolar II) and III (Model II plus recurrent unipolar individuals).

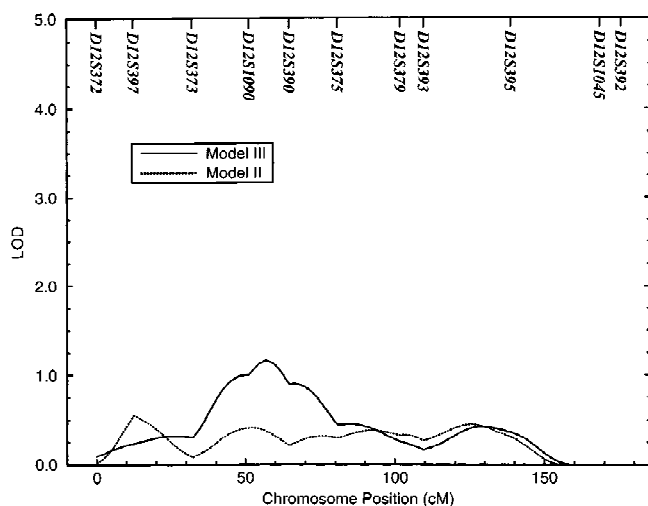


Fig. 5. Multipoint lod score for chromosome 12. Results are computed using IBD information on all possible pairs of affected siblings for diagnostic Models II (schizoaffective bipolar, bipolar I and bipolar II) and III (Model II plus recurrent unipolar individuals).

tibility loci and the power to detect each individual locus was 0.2, the power to detect at least one would be 80%. However, a replication study for that particular locus would still only have 20% power. This problem is compounded when several diagnostic schema are used so that multiple, non-independent tests contribute to the probability of chance detection.

The linkage reports for the APO-E findings in late-onset Alzheimer's disease had lod scores of approximately 2, but subsequent studies have provided consistent evidence for an elevated risk to those carrying the  $\epsilon 4$  allele. If there are comparable genes for the susceptibility to bipolar illness, they may be hard to detect with the currently available sample sizes. MOD scores above 3 can arise due to chance [Rice et al., 1995], and it is always possible that the largest effects observed in a genome screen may be false positives, while the *true* signals may only give modest evidence. Genotyping of additional markers around our hot spots should clarify the importance of some of these regions. As noted by Kruglyak and Lander [1995], a 10 cM map only extracts approximately 70% of the total information about IBD status at a marker and approximately 60% between markers.

## ACKNOWLEDGMENTS

Some of the results of this paper were obtained by using the program package S.A.G.E., which is supported by a U.S. Public Health Service Resource Grant (1 P41 RR03655) from the Division of Research Resources. AMG is the recipient of an NIA career development award AG99534.

## REFERENCES

Baron M (1996): Linkage results in schizophrenia. *Am J Med Genet* 67:121–123.  
 Blackwelder WC, Elston RC (1985): A comparison of sib pair linkages tests for disease susceptibility loci. *Genet Epidemiol* 2:85–97.

Blehar MC, Weissman MM, Gershon ES, Hirschfeld RMA (1988): Family and genetic studies of affective disorders. *Arch Gen Psych* 45:289–292.  
 Boehnke M (1991): Allele frequency estimation from pedigree data. *Amer J Hum Genet* 48:22–25.  
 Clerget-Darpoux F, Bonaiti-Pellie C, Hochez J (1986): Effects of misspecifying genetic parameters in lod score analysis.  
 Craddock N, McGuffin P (1993): Approaches to the genetics of bipolar disorders. *Ann Med* 25:317–322.  
 Craddock N, Krishnan VHR, Prasher VP, Davison V (1993): Major affective disorder associated with duplication of distal long arm of chromosome 11. *Psychiatr Genet* 3:149.  
 Craddock N, Owen M, Burge S, Kurian B, Thomsas P, McGuffin P (1994): Familial cosegregation of major affective disorder and Darier's disease (keitosis folliu(sis). *Br J Psychiatry* 164:355–358.  
 Dawson E, Parfitt E, Roberts Q, Daniels J et al (1995): Linkage studies of bipolar disorder in the region of the Darier's disease gene on chromosome 12q23-24.1 *Am J Med Genet (Neuropsychiatric Genetics)* 60:94–102.  
 Dawson E, Gill M, Curtis D, Castle D, Hunt N, Murray R, Powell J (1995): Genetic association between alleles of pancreatic phospholipase A2 gene and bipolar affective disorder. *Psychiatric Genet* 5:177–180.  
 Elston RC (1989): Man bites dog? The validity of maximizing lod scores to determine mode of inheritances. *Am J Med Genet* 34:487–488.  
 Ewald H, Mors O, Flint T, Kruse TA (1994): Linkage analysis between manic depressive illness and the region on chromosome 12q involved in Darier's disease. *Psychiatric Genet* 4:195–200.  
 Kendler KS, Straub RE, MacLean CJ, Walsh D (1996): Reflections on the evidence for a vulnerability locus for schizophrenia on chromosome 6p24–22. *Am J Med Genet* 67:124–126.  
 LaBuda MC, Maldonado M, Marshall D, Otten K, Gerhard DS (1996): A follow-up report of a genome search for affective disorder predisposition loci in the Old Order Amish. *Am J Hum Genet* 59:1343–1362.  
 Mowry et al (1995): Schizophrenia susceptibility and chromosome 6p24–22. *Nature Genetics* 11:233–236.  
 NIMH Genetics Initiative Bipolar Group (1997): Genomic survey of bipolar illness in the NIMH genetics initiative pedigrees: A preliminary report. *Am J Med Genet* 74:227–237.  
 Ott J (1991): Analysis of human genetic linkage. The Johns Hopkins University Press, Baltimore, MD.  
 Rice JP, Neuman RJ, Hoshaw SL, Daw EW, Gu C (1995): TDT with covariates and genomic screens and mod scores: Their behavior on simulated data.  
 Rice JP, Bolton M, Shears S, Wu W, Goate A (1995): Scoring of genotypes from multiplexed fluorescently labelled markers. Abstract. *Am J Hum Genet*:A201.  
 Risch N (1984): Segregation analysis incorporating linkage markers. I. Single locus models with an application to Type I diabetes. *Am J Hum Genet* 36:363–386.  
 Risch N, Botstein D (1996): A manic depressive history. *Nat Genet* 12:351–353.  
 S.A.G.E. (1994): Statistical Analysis for Genetic Epidemiology, Release 2.2. Computer program package available from the Department of Biometry and Genetics, LSU Medical Center, New Orleans.  
 Schwab SG, Albus M, Hallmayer J, Honig S et al (1995): Evaluation of a susceptibility gene for schizophrenia on chromosome 6p by multipoint affected sib-pair linkage analysis. *Nat Genetics* 11:325–327.  
 Sham PC, Morton NE, Rice JP (1992): Segregation analysis of the NIMH collaborative study family data on bipolar disorder. *Psych Genet* 2:175–184.  
 Straub RE, MacLean CJ, O'Neill FA, Burke J, et al (1995): A potential vulnerability locus for schizophrenia on chromosome 6p24–22: evidence for genetic heterogeneity. *Nat Genetics* 11:287–293.  
 Taylor MA, Berenbaum SA, Jampala VC, Cloninger CR (1993): Are schizophrenia and affective disorder related? Preliminary data from a family study. *Am J of Psychiatry* 150(2):278–285.  
 Turecki G, Smith MdeAC, Mari JJ (1995): Type I bipolar disorder associated with a fragile site on chromosome 1. *Am J Med Genet* 60:179–182.  
 Wang S, Sun C, Walczak CA, Ziegler JS et al (1995): Evidence for a susceptibility locus for schizophrenia on chromosome 6pter–p22. *Nature Genet* 10:41–46.